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INTERCHANGE OF GROUP C BETA HEMOLYTIC STREPTOCOCCI AMONG DOGS AND MONKEYS CONFINED IN DYNAMIC FLOW ATMOSPHERIC SYSTEMS

JAMES G. KING, III, TECHNICAL SERGEANT, USAF

SEPTEMBER 1967

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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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Foreword

This research was conducted in the Pathology Branch, Toxic Hazards Division, Biomedical Laboratory of the Aerospace Medical Research Laboratories. The work was performed in support of project 6302, Toxic Hazards of Propellants and Material; task 630206, Toxicological Support. Altitude facility maintenance and experimental animal care were provided by Aerojet-General Corporation, Azusa, California, under Contract AF 33(657)-11305. This work was partially funded under NASA Defense PRT-31248G. The author wishes to express gratitude for the assistance and encouragement of Dr. David T. Harper Jr. and Dr. Farrel R. Robinson. The author also wishes to thank A1C Will A. Knight Jr. for assistance in the experimental work.

This technical report has been reviewed and is approved.

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Abstract

Serial throat and rectal bacteriological samples were taken from closely confined animals in dynamic flow atmospheric chambers during toxicology experiments at both altitude and ambient conditions to measure the extent of bacterial interchange in environments similar to those in space cabins. Dogs and monkeys were exposed for prolonged periods to 0.2 mg/m^3 ozone in air at 740 mm Hg, to 100% oxygen at 260 mm Hg, and to ambient conditions. The incidence of isolation of Lancefield group C beta hemolytic streptococci increased from 25 to 91%. Because of this increased rate of isolation of serologically uniform beta hemolytic streptococci, transfer of this organism between animals is postulated. The effects of confinement on bacterial interchange in this particular set of atmospheres are discussed.

SECTION I.
Introduction

The transmissibility of microorganisms among individuals in a closed environment is dependent upon numerous factors among which are the defense mechanisms of the host, virulence, and viability of the organism involved, method of transmission, temperature, humidity, and the characteristics of air flow (ref 4).

Interest in the transmission of microorganisms among individuals within confined atmospheric systems has been stimulated by studies of population dynamics of bacterial flora in such systems, problems in sterilizing space vehicles, and possible effects of potentially pathogenic bacteria on man during prolonged space flights.

Under space-cabin conditions, man will not only be exposed to close personal contact with other crewmembers, but will also be denied many routine personal hygiene procedures (ref 3). Because of the physical and mental stresses to which he will be subjected, his defense mechanisms may well be lowered (ref 6). Dogs and monkeys in this study were subjected to environmental conditions in altitude and ground level chambers approximating those to be experienced by man in space.

SECTION II.
Exposure Conditions

These experiments were conducted on beagles and monkeys *(Macaca mulatta)* during inhalation toxicology experiments at the Aerospace Medical Research Laboratories. Experimental animals were exposed in Thomas domes, which are dynamic flow altitude chambers having a total air volume of approximately 870 ft^3, capable of maintaining constant temperature, relative humidity, total pressure, air flow, and atmospheric composition for prolonged periods with or without introduction of contaminant (ref 5). Control animals were concurrently maintained in Rochester chambers, hermetically sealed ground-level chambers having controlled temperature, humidity, air flow and contaminant concentration, and total air volume of approximately 75 ft^3.

In experiment I, eight beagles and four monkeys were exposed in a Thomas dome for 90 days to 0.2 mg/m^3 ozone in air at 740 mm Hg total pressure (table I). In experiment II in another Thomas dome, a similar group of animals was exposed simultaneously for 235 days to 100% oxygen at 260 mm Hg pressure. Control animals in the Rochester chambers were maintained at approximately 740 mm Hg in air. Temperature in all units was maintained at approximately 23 C. Relative humidity averaged about 70% in the Thomas domes, and about 50% in the Rochester chambers. Initially, air flow within the Thomas domes was approximately 20±2 ft^3/min, with increases to about 30 ft^3/min when the dome was entered by maintenance and cleaning personnel. The air flow in experiment I was raised to 32 ft^3/min on the 34th day, and to 42 ft^3/min on the 38th day in an attempt to lower the relative humidity. It was maintained at 42 ft^3/min for the remainder of the experiment. The air flow within the Rochester chambers was approximately 32 ft^3/min throughout the study.

TABLE I

EXPERIMENTAL CONDITIONS

	Thomas Dome I Experiment 1	*Thomas Dome IV Experiment 2*	*Rochester Chambers Controls 1 & 2*
Temperature	23°C ± 3	23°C ± 3	21°-24°C
Relative Humidity	66-78%	66-78%	50%
Gas Atmospheric Flow	20 ± 2 ft^3/min (32 ft^3/min—Days 34-38) (42 ft^3/min—Days 38-40)	20 ± 2 ft^3/min	32 ft^3/min
Pressure	740 mm Hg	260 mm Hg	740 mm Hg
Basic Atmosphere	Air	100% O_2	Air
Contaminant	0.2 mg/m^3 O_3	None	None
No. of Dogs	8	8	8
No. of Monkeys	4	4	0
Duration of Experiment	90 Days	235 Days	Experiment 1: 90 Days Experiment 2: 235 Days
Chamber Volume	870 ft^3	870 ft^3	75 ft^3

CONDITIONS OF ANIMAL CONFINEMENT AND CARE

Dogs in the Thomas domes had approximately 4.5 ft^2 of floor space per animal; those in the Rochester chambers had about 2.5 ft^2 per animal. The monkeys in the Thomas domes had about 2 ft^2 of floor space for each animal. Figures 1 and 2 are presented to illustrate the physical confinements and the locations of each animal. Control female beagles for experiments I and II were confined in one Rochester chamber, while the male controls for both experiments were confined in the other Rochester chamber. The controls for experiment I were confined with the controls for experiment II on day 118 of experiment II.

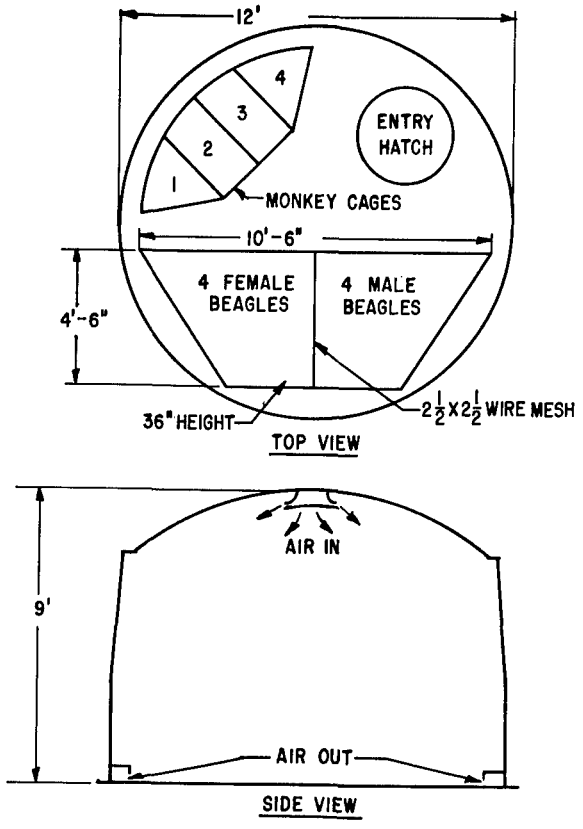

Figure 1. Thomas Dome Configuration and Animal Placement.

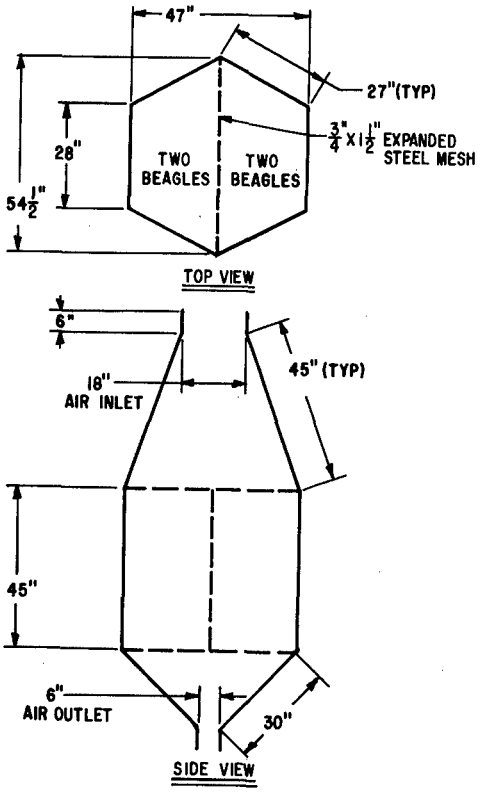

Figure 2. Rochester Chamber Configuration and Animal Placement.

Oral contact and inhalation of respiratory aerosols were possible among all beagles confined in the same chamber or dome. Only wire mesh separated the groups of dogs within each unit. Physical contact was not possible between singly caged experimental monkeys, but close placement of cages allowed inhalation of respiratory aerosols.

Data from the four control monkeys for experiments I and II were not included in the tables because they were maintained in cages in an open animal room. However, bacterial isolations made from this last group showed normal flora. Standard laboratory diet and water were available to the animals ad libitum. Cages and chamber floors were cleaned daily with water. All food and water pans were replaced with clean pans daily.

SECTION III.
Bacteriological Sampling

Throat and rectal bacteriological samples were collected from each animal, biweekly in experiment I and monthly in experiment II. Blood samples were taken at the same times as the bacteriological samples. Sampling in experiment II was scheduled monthly to reduce blood loss from the animals during the 235-day experiment. The cultures from all animals were observed closely for any increase in incidence of microorganisms which had been harbored by other animals within the same chamber. By observing closely the identity of these "marker" organisms and the incidence of their isolation, the extent of bacterial transfer among these animals was assessed.

THROAT CULTURES

Throat samples were taken with sterile cotton swabs from the peritonsillar area. The swabs were then placed in a tube containing approximately 1 ml of nutrient broth.* This was necessary because of excessive drying of the samples during the 2-3 hours required to complete the procedures within the domes, remove the samples, and inoculate them onto plates. The samples were inoculated as soon as possible on 5% blood agar* and eosin methylene blue (EMB) agar* and incubated for 24 hours at 37C. All organisms isolated were identified as to genus and to species where time permitted. To confirm the nature of all organisms identified as beta hemolytic streptococci, gram stains were performed and all were typed into Lancefield groups. BBL Taxos A discs* containing 0.02 units of Bacitracin were used to identify group A organisims. BBL SF medium* (selective media for group D) was used for identification of that group. The Camp test (ref 1), a streak plate method involving beta lysin-producing *Staphlyoccus aureus,* was used for identification of group B organisms. Beta hemolytic streptococci negative to all these tests were considered to be group C. Because approximately 2.6% of group A streptococci are not inhibited by Taxos A discs, representative cultures of isolated beta hemolytic streptococci negative to all these tests were maintained until serological typing could be performed. These representative isolates were then confirmed to be group C with the use of typing sera.

RECTAL CULTURES

Rectal samples were collected by inserting a sterile cotton swab approximately 5 mm into the animal's rectum. The swabs were then placed in a tube containing approximately 1 ml of 1% Peptone* and were subsequently inoculated on Salmonella-Shigella (SS) agar* and EMB agar*, and placed into tubes containing 7-8 ml of Selenite-F broth*. The plates and broth were then incubated for 24 hours at 37C. At the end of 24 hours, the swabs in the Selenite-F broth were inoculated on SS and EMB agar and incubated at 37C for 24 hours. Organisms isolated on all plates were identified by colony characteristics on SS and EMB agar and by routine biochemical reactions. Results were evaluated for potential pathogens which might serve as "marker" organisms for evaluating any possibility of bacterial interchange among animals.

*Baltimore Biological Laboratory Division, 2201 Aisquith St., Baltimore, Maryland.

SECTION IV.
Results

This study showed a definite increase in the incidence of group C beta hemolytic streptococci isolated during the course of experiments I and II, and the concurrent control groups (tables II, III and IV).

Among all animals confined in the Thomas dome for experiment I, beta hemolytic streptococci were isolated from the preexposure throat samples of only two dogs. An additional isolation was made from beagle AB-40 on days 16 and 31 of the experiment. By the 44th day of the experiment, after two increases in air flow in this chamber, this organism had been isolated from seven of eight dogs and two of four monkeys. During the remainder of experiment I, group C beta hemolytic streptococci was isolated from every other dog, and from a 3rd monkey (table II). One dog, AB-21, which had been a preexposure carrier of this organism, died on the 45th day of experiment with gross evidence of ozone toxicity, including pulmonary congestion, hemorrhage and edema. Microscopic lesions in the lungs confirmed hemorrhage and edema, but no evidence of pneumonitis or an infectious inflammatory process was found.

TABLE II

INCIDENCE OF LANCEFIELD GROUP C BETA HEMOLYTIC STREPTOCOCCI

Experiment I

THOMAS DOME No. 1

DOGS	*Preexposure*	*16 Day*	*31 Day*	*44 Day*	*58 Day*	*73 Day*	*87 Day*
AB-38 (M)	—	—	—	+*	+	+	+*
AB-40 (M)	—	+*	+*	+	+	+*	+
AB-52 (M)	—	—	—	+	+	—	+
AB-44 (M)	—	—	—	+	+	—	+
AB-05 (F)	—	—	—	+	+*	+	+
AB-11 (F)	+	—	—	+*	+	+	+*
AB-21 (F)	+*	—	—	+*	(Died day 45)		
AB-25 (F)	—	—	—	—	+	+	—
MONKEYS							
B-18 (M)	—	—	—	+	—	+	—
A-98 (M)	—	—	—	—	+	—	+*
A-65 (F)	—	—	—	—	—	—	—
A-79 (F)	—	—	—	+*	—	—	+

*Lancefield Group C Beta Streptococci was predominant organism isolated.
−Negative for Lancefield Group C Beta Hemolytic Streptococci.
+Positive for Lancefield Group C Beta Hemolytic Streptococci.

(F) Female
(M)Male

The isolations in experiment II have a scattered pattern and are not as obviously time-related as in experiment I. The first samples from these animals were not taken until the 79th day of experiment, because microbiological sampling was not part of the toxicological investigation until that time. In the initial cultures from this experiment, group C beta hemolytic streptococci were isolated from four of the eight beagles and none of the monkeys. During the remainder of the experiment, however, this organism was isolated at one time or another from all dogs and from two out of the four monkeys (table III).

TABLE III

INCIDENCE OF LANCEFIELD GROUP C BETA HEMOLYTIC STREPTOCOCCI

Experiment II

THOMAS DOME No. 4

DOGS	*79 Day*	*107 Day*	*135 Day*	*163 Day*	*191 Day*
CF-3 (M)	—	—	—	—	+*
CI-1 (M)	—	—	—	+*	+
CE-1 (M)	+*	+*	—	+	—
CM-1 (M)	—	+	—	—	+
CP-2 (F)	+*	+	—	—	—
CJ-1 (F)	+*	—	+	+	+*
CI-6 (F)	—	—	—	—	+
CH-3 (F)	+*	+*	+	+*	+*
MONKEYS					
A-54 (M)	—	—	—	—	—
A-62 (M)	—	—	—	—	+
A-39 (F)	—	+*	—	—	—
A-25 (F)	—	—	—	—	—

*Lancefield Group C Beta Streptococci was predominant organism isolated.
—Negative for Lancefield Group C Beta Hemolytic Streptococci.
+Positive for Lancefield Group C Beta Hemolytic Streptococci.

(F) Female
(M) Male

Group C beta hemolytic streptococci were isolated from the initial cultures from two control dogs, one male and one female, which were confined in the Rochester chambers. No beta hemolytic streptococci were isolated from the experiment I controls before their confinement with the experiment II controls. During the course of the two experiments, group C beta hemolytic streptococci were isolated at least once from all male and female controls as shown in table IV.

Except for the beta hemolytic streptococci, all animals in these experiments maintained throat flora which, in comparison with unpublished data in this laboratory, were regarded as essentially normal.

The rectal cultures revealed no organisms which could be used as "marker" organisms during this study. Rectal flora consisted of combinations of *Escherichia coli, Aerobacter cloacae* and *Proteus* species.

TABLE IV

INCIDENCE OF LANCEFIELD GROUP C BETA HEMOLYTIC STREPTOCOCCI

Control Dogs for Experiment I & II

ROCHESTER CHAMBER No. 1 & 2

	Animal	*Preexposure*	*16 Day*	*31 Day*	*44 Day*	*58 Day*	*73 Day*	*87 Day*
EXPERIMENT	AB-24 (M)	—	+	—	—	+	+	+
No. 1	AB-28 (M)	—	—	+*	—	+	—	—
CONTROLS	AB-13 (F)	—	—	+*	+*	+	+	+*
	AB-15 (F)	—	—	+*	+	+	+	—

	Animal	*79 Day*	*107 Day*	*135 Day*	*163 Day*	*191 Day*
EXPERIMENT	CP-1 (M)	+	+	—	+*	—
No. 2	CP-2 (M)	—	—	—	+*	—
CONTROLS	CH-4 (F)	—	—	+*	+*	+
	CJ-2 (F)	+*	—	+	+*	+*

*Lancefield Group C Beta Streptococci was predominant organism isolated.
– Negative for Lancefield Group C Beta Hemolytic Streptococci.
+ Positive for Lancefield Group C Beta Hemolytic Streptococci.

(F) Female
(M) Male

Attempts were made to correlate total white blood cells (WBC) and total immature neutrophils with the incidence of beta hemolytic streptococci. There was no correlation of elevation of the total WBC or shift of the neutrophil percentage coinciding with the occurrence of beta hemolytic streptococci in cultures from any animal.

The animals were killed with an overdose of intravenous sodium pentobarbital at the termination of both experiments, and gross and microscopic examinations were performed. There was no gross or microscopic evidence of pneumonitis, bronchitis or other infectious inflammatory process. Pharyngeal lymphoid tissue was not examined microscopically.

Eight other experiments, involving exposures of 90 days or longer to various atmospheres and contaminants at ambient and altitude conditions have been completed at this laboratory since monitoring of microbiological flora was started. Atmospheric and confinement conditions were similar to those in the experiments presented in this study. Data from the other eight experiments showed no significant increase in incidence of any organism. "Marker" organisms followed during these eight experiments included beta hemolytic streptococci, hemolytic *Staphlyococcus aureus* and *Klebsiella pneumoniae*. Since the incidence of these organisms did not increase or cause disease, they were not typed or otherwise classified. These streptococcal isolates were not pathogenic nor did they spread among cage mates. Air flow rates were maintained in the range from 20 to 25 ft^3/min as in experiment II whereas in experiment I the air flow was progressively increased on two occasions to a peak of 42 ft^3/min.

SECTION V.
Discussion

The data obtained from these experiments indicate a definite increase in the incidence of isolation of group C beta hemolytic streptococci from animals confined in dynamic flow atmospheric systems during exposure to ozone in ambient air, to 100% oxygen at altitude and to ambient air alone. In experiment I, the greatest increase in incidence of group C beta hemolytic streptococci was preceded by two successive increases in air flow. Increases in air turbulence associated with these changes in air flow may well have increased the movement of airborne bacteria within the dome. A similar increase in the incidence of this organism occurred among the control dogs confined in the Rochester chambers at about the same time. The air flow within these chambers was constant throughout the study, but was at a high rate. The incidence of isolation of group C beta hemolytic streptococci in experiment II did not reach a peak as it did in experiment I and the control group. There seemed to be a more generalized, transient carrier state in these animals. Compared with the other two chambers, air flow was uniform at a lower rate in this chamber.

The appearance of the group C beta hemolytic streptococci in the monkeys is particularly significant as there was no possibility of physical contact among monkeys or between monkeys and dogs. In experiment I, no isolations of this organism were made from any monkey until day 44, after the airflow had been increased. Thereafter at least one isolation was made from these four animals on each sampling date. The monkeys could have picked up the organism through airborne transmission or from cages contaminated by personnel who had cleaned dog cages previously, but the time sequence indicates the probability of the first mechanism being operative. Chamber maintenance and operational techniques were unvarying throughout the exposure period.

All dogs, on the other hand, had ample opportunity at all times for physical contact with other dogs infected with group C beta hemolytic streptococci.

Several factors acting independently or in combination may explain the sporadic recovery of beta hemolytic streptococci in experiment II. Sampling techniques were well standardized, although sampling intervals may have coincided with low ebbs of streptococcal populations and the organisms may not have been isolated. The variable introduced by inapparent infections in the people entering the chamber is difficult to assess. The typing of the organism into one Lancefield group would tend to discount the introduction of the same type of organism by several different people on many different occasions. The length of time involved in these experiments would tend to eliminate the possibility of the organism being brought in continually in numerous sacks of feed from several different shipments. Feed from the same sources has been used in many other experiments in this laboratory and no such organisms have been isolated from dogs and monkeys sampled bacteriologically as those reported above. The water itself was not a likely source since it was obtained from a large chlorinated supply of the Air Force Base, but the common dog watering pans within 4-dog cages may have served as fomites by temporarily harboring the organism. The monkeys had individual automatic waterers.

The increase in isolation of beta hemolytic streptococci in experiment I, (table II), was possibly caused by the ozone contaminant. Ehrlich (ref 2) has shown a definite increase in susceptibility to bacterial infection after exposure to ozone.

The data indicate the presence of intermittent carrier states in two or more animals in each experiment. As a follow-up study, it would be particularly interesting and pertinent to perform an experiment similar to these using only one animal infected in the laboratory with a preselected

known strain of a pathogenic organism as the sole transfer source for a group of animals proved free of this organism before introduction into the chamber.

It would have been desirable to type serologically all beta hemolytic streptococci isolated. At the time however, typing sera was unavailable and it was impractical to maintain a stock culture of all isolations. All beta hemolytic streptococci isolated correspond to the criteria for Lancefield group C by the method used and it is doubtful that any of the isolates were of another group.

It would have been desirable to take bacteriological samples from the animals more often, but limitations of personnel and altitude chamber entrance procedures demanded that the samples be taken simultaneously with blood drawing for blood chemistry determinations.

The definite increase in the incidence of group C beta hemolytic streptococci in the absence of other groups would indicate that transfer of organisms of this group among these animals did occur. This transfer occurred among all animals that had direct physical contact with animals harboring this organism, and to a lesser degree among those exposed to the respiratory aerosols of carrier animals.

On the basis of these studies, it would seem advisable to maintain turbulence free, low air flow rates in manned space cabins and in simulator environments, and to screen all experimental subjects used in experiments of this type for potentially pathogenic microorganisms.

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13. ABSTRACT

Serial throat and rectal bacteriological samples were taken from closely confined animals in dynamic flow atmospheric chambers during toxicology experiments at both altitude and ambient conditions to measure the extent of bacterial interchange in environments similar to those in space cabins. Dogs and monkeys were exposed for prolonged periods to 0.2 mg/m^3 ozone in air at 740 mm Hg, to 100% oxygen at 260 mm Hg, and to ambient conditions. The incidence of isolation of Lancefield group C beta hemolytic streptococci increased from 25 to 91%. Because of this increased rate of isolation of serologically uniform beta hemolytic streptococci, transfer of this organism between animals is postulated. The effects of confinement on bacterial interchange in this particular set of atmospheres are discussed.

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